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REPORT

ON THE

ACTION OF PAPAIN.

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REPORT ON THE ACTION OF PAPAIN.

In a previous paper (*Journal of Physiology*, vol. v., No. 4) I have detailed the characters and action on coagulated albumen of the proteolytic ferment obtained from the papaw-juice (Carica papaya) extending the researches of Wurtz and Bouchut and others.

Wurtz had described the ferment as a proteid, soluble in distilled water, yet precipitated by nitric acid, but differing from a native albumen (as white of egg) in not being precipitated by boiling. In the material I used in my former experiments (commercial papain), I found two proteids, a globulin, and a "peptone;" and I could not come to any conclusion as to which of these bodies was the ferment, or, to speak more correctly, which was associated with it.

In the present investigation, I attempted to settle this point. In the first place, a body called a "peptone" in my previous paper is not a true peptone—that is, a proteid capable of fairly rapid diffusion, not precipitated by nitric nor by acetic acid and ferrocyanide of potassium; but it is one of the bodies intermediate between globulins and peptone, first described by Meissner as a-peptone, and called by Kühne *hemialbumose*. This body agrees with peptone in the following reactions: it is soluble in distilled water, and is not precipitated from this solution by boiling; it also gives a pink or red colour with copper-sulphate and excess of potash. It differs from peptones in being precipitated by strong mineral acids, and by acetic acid and ferrocyanide of potassium. These reactions agree with those given by Wurtz as characteristic of solutions of pure papain; this agreement, indeed, led me to think that the ferment was

associated with the hemialbumose. I found this to be the case. A glycerine extract was made of commercial papain, the glycerine being filtered clear under pressure. This extract contained a proteid (hemialbumose) in quantity, and a mere trace of globulin. It was as active as the powder itself. Part of this extract was diluted with water, and saturated with magnesium sulphate to precipitate the small amount of globulin, which was filtered off; the filtrate was then saturated with sodium-sulphate, which precipitated the hemialbumose. This was collected on a filter, washed with a saturated solution of sodio-magnesium-sulphate, and then dissolved in water. This solution of the precipitated hemialbumose was found to be very active; it was tested with coagulated egg-albumen, peptones being formed in quantity. The filtrate, after saturation with sodium-sulphate, contained a little hemialbumose. After dialysing for some hours, its action was tested on egg-albumen; very little, if any, digested. This experiment distinctly shows that the ferment action is associated with the hemialbumose.

The result was confirmed in another experiment, in which a similar process of saturation was performed in a watery solution of papain. The result may be tabulated as follows.

Precipitate by Magnesium Sulphate = Globulin.	Precipitate by Sodium Sulphate=Hemialbumose	Filtrate containing no Proteid.
No action on coagulated egg-albumen at 35° to 40° Cent.	Forms peptones from coagulated egg-albumen at 35° to 40° Cent.	No action on albumen at 35° to 40° Cent.

Whether the ferment may be separated from the hemialbumose I am at present unable to state. Ptyalin (Cohnheim) and pepsin (Brücke) have been separated free from proteid. Trypsin, however, has not, though Schutzenberger states that probably all diastatic and proteolytic ferments may be separated from the accompanying proteids.

I have repeated the experiments on animal albumen detailed in my first paper, and can only confirm what I

have there stated; namely, that papain acts like trypsin (though not so rapidly) in forming from coagulated albumen and fibrin a true peptone, an intermediate body related to globulin, and leucin and tyrosin.

I have extended my experiments to the investigation of the action of the ferment on milk, and on the proteids found in papaw-juice.

Action on Milk.—Papain acts like pancreatic juice on milk, and the experiments I shall describe are almost similar to those performed by Dr. W. Roberts, of Manchester, with pancreatic extract. Papain, like pancreatic extract, first curdles the milk, and, within certain limits of temperature, the curds are more quickly formed and are larger the higher the temperature up to 62° Cent. (about 145° Fahr.), at which point the curdling is practically instantaneous; for example, with five grains of papain, and 450 cubic centimètres of milk, and 125 cubic centimètres of water at 62° Cent.

The curdling is hindered by making the milk alkaline with bicarbonate of soda, by diluting it, and also to some extent by boiling the milk previously to the addition of an equal quantity of cold water; when, if papain be added, the curdling is not so great, nor the curds so large, as when the water is boiled and added to the milk. The curds in "papainised" milk gradually dissolve, the casein being changed into peptones, leucin and tyrosin being produced, and the liquid becoming bitter to the taste. Moreover, between the stage of casein and peptone there is a body formed, which is precipitated by boiling and by nitric acid, an intermediate body similar to the one developed during the digestion of coagulated egg-albumen. Its properties were tested as follows. Seeing that it must be formed from the curds first precipitated by the ferment, these were separated in one experiment, and extracted with a ten per cent. sodium-chloride solution, and the mixture filtered. The clear filtrate gave a fine precipitate on boiling, and on adding nitric acid; and, moreover, a fairly copious one on saturation with sodium-chloride. This last precipitate was collected and dissolved in water (by aid of the salt present), and gave

the following reactions, in addition to those previously obtained from the unsaturated filrate—namely, a marked biuret reaction with copper-sulphate and potash, and a cloudiness with corrosive sublimate, insoluble in excess; boiled with fresh ferric acetate and filtered, no proteid was found in the filtrate, showing the absence of peptones. Hence this body, which is soluble in saline solutions, and precipitated from these by saturation with sodium-chloride, and giving a biuret reaction, is a hemialbumose.

This point being settled, experiments were done to see the degree of action of the ferment in the milk. In the following experiment, the digestion of the curds (casein and fat) obtained by precipitating 200 cubic centimètres of milk diluted, with glacial acetic acid, was compared with that of the same quantity of milk. The curd was well washed to free from acid, and squeezed as dry as possible before weighing.

A.

Milk	200 cubic centimètres.
Sodic carbonate ...	.5 gramme (7½ grains).
Water	200 cubic centimètres.
Papain	.3 gramme (5 grains).

The water and sodic carbonate were boiled and added to the milk (which was at 10° Cent.); resulting temperature 50° Cent. The papain was then stirred in the beaker, which was wrapped up and kept in a warm place. In 10 minutes the mixture began to curdle, the curds gradually dissolving; in 45 minutes a slight bitter taste was developed; in 50 minutes the temperature of the liquid was 35° Cent. It was then boiled, causing a slight precipitate. The filtered liquid gave the tests for peptones.

B.

Curd prepared as above from 200 cubic centimètres of milk; weight ...	21.5 grammes.
Water	200 cubic centimètres.
Sodic carbonate	.5 gramme (7½ grains).
Papain	.5 gramme (7½ grains).

Half the water was boiled and added to the other half

containing the curd and sodic carbonate; resulting temperature 48° Cent. It was placed in a warm place under cover for 65 minutes, when the residue of curd weighed only 2.7 grammes; therefore (21.5-2.7) = 18.8 grammes digested. The residue was chiefly fat; it dissolved almost completely in ether. The filtrate after digestion gave a slight precipitate with acetic acid in the cold, soluble in excess; none on boiling; a marked biuret reaction with copper-sulphate and potash.

It will be noticed that A was partly digested, giving a precipitate on boiling; B almost completely so, since there was no precipitate on boiling. The precipitate by acetic acid, soluble in excess, was hemialbumose. Both A and B were slightly bitter after digestion.

The point naturally suggested by these experiments was, that papain might be utilised in preparing an artificial peptonised milk, its slower action being in some respects an advantage over pancreatic extract, in that the digestion can be arrested at any intermediate stage more readily. In some conditions of disease, it seems to me a distinct advantage to employ a partly digested food, because some work is left for the stomach to accomplish; in others, perhaps, a fully peptonised food would be more useful.

By a partly digested milk is meant one in which much of the casein is in an intermediate stage—namely, as "metacasein" and hemialbumose: by a fully digested milk, one where all of the casein has been changed into peptone. A and B, in the experiment quoted above, are types of the two stages.

Milk which has undergone only partial digestion is not very bitter, but has the disadvantage that it causes a precipitate on boiling afterwards. This latter result may be obviated by making it sufficiently alkaline—that is, adding 30 or 40 grains of bicarbonate of soda to the pint of milk. It is only slightly different in appearance from ordinary milk. The wholly digested milk is more bitter.

The following practical suggestions may be made regarding the preparation of papainised milk.

A pint of milk is taken, and a quarter of a pint of water; add an equal volume of milk to the water, and 30 grains of bicarbonate of soda, and boil; add the remaining milk to the hot liquid. The resulting temperature varies from 45° to 55° Cent.; it is usually about 48° Cent. (118° Fahr.); the variation depends, of course, on the temperature of the cold milk. The papain must now be quickly stirred in, and the mixture covered with a cosey, and placed in a warm place. After digestion, it is boiled to stop the action. This method does as well for pancreatic as for papain digestion; it obviates the use of a thermometer, and so can readily be done in the ward or sick-room.

For preparing a partly digested milk, seven grains of papain, with an hour and a half's digestion, is quite sufficient, using a pint of milk in the manner above described; for the more complete digestion, 10 grains for two hours must be used.

The food is greedily taken by kittens, but I have not yet tried it on patients.

Action of Papain on the Proteids in Papaw-Juice.—(Only a brief summary of the results attained can now be given; full details of the experiments will soon be published.) Of late years the former ideas of the nature and constitution of vegetable proteids have been entirely revolutionised, chiefly by the researches of Denis (*Mémoire sur le Sang*), Weyl, Hoppe-Seyler, Vines, and others; so that now we may state that the two chief proteids found in plants are globulins and "peptones." Vines considers that there is no true peptone in the seeds of plants; he thinks it is a hemialbumose, and explains away Ritthausen's "legumin" and "conglutin," obtained from the seeds of Leguminosæ, referring the former to the class of hemialbumoses, and the latter to a changed form of proteid produced by the action of alkalies on globulin. (*Proc. Roy. Soc.*, vol. xxviii., 1878.) By pursuing the method first instituted by Denis—namely, extracting the material with 10 to 15 per cent. solution of sodium-chloride, and precipitating the proteids by saturation with salts, I have obtained from papaw-juice proteid bodies whose reac-

tions agree with those of globulins and hemialbumoses, or rather albumoses, leaving the question as to whether they are anti- or hemi- forms for further consideration. The salts used in saturating were *magnesium-sulphate*, which precipitated the globulin of the myosin type and one form of albumose; followed by *sodium-sulphate*, which, by forming the double salt sodio-magnesium sulphate, precipitated the remaining proteids, which consisted of a trace of coagulable proteid and an albumose (Kühne, Ueber Albumosen, *Zeitschr. für Biologie*, Band xx., 1884).

The albumose precipitated by sodio-magnesium sulphate corresponds to Vines' hemialbumose; its exact position I must leave for the present undetermined. This albumose gives the same reactions previously detailed, as those of the body with which the ferment is so closely associated; it is the proteid in the juice most like a peptone. I found no true peptone.

The action of papain on these different constituents is peculiar, because in the many experiments I have hitherto done, I have been able to discover no true peptone as a result of digestion; the body which is formed from the globulins is the albumose found in small quantities in the salt extract, the body which corresponds to Vines' hemialbumose.

At the same time leucin and tyrosin are formed from these proteids; they are found in the juice as well.